they bind to the target nucleic acid as illustrated in Figures 15A-G. In the formats shown in Figure 13A-B and 18, the binding oligonucleotides may be used to bring the acceptor and donor fluorescent molecules on the two nanoparticles in proximity. Also, in the format illustrated in Figure 13A, the oligonucleotides attached the substrate may be labeled with **d**. Further, other labels besides fluorescent molecules can be used, such as chemiluminescent molecules, which will give a detectable signal or a change in detectable signal upon hybridization.

Another embodiment of the detection method of the invention is a very sensitive system that utilizes detection of changes in fluorescence and color (illustrated in Figure 21). This system employs latex microspheres to which are attached oligonucleotides labeled with a fluorescent molecule and gold nanoparticles to which are attached oligonucleotides. The oligonucleotide-nanoparticle conjugates can be prepared as described above. Methods of attaching oligonucleotides to latex microspheres are well known (see, *e.g.*, Charreyre et al., *Langmuir*, **13**:3103-3110 (1997); Elaissari et al., *J. Colloid Interface Sci.*, **202**:251-260 (1998)), as are methods of labeling oligonucleotides with fluorescent molecules (see above). The oligonucleotides on the latex microspheres and the oligonucleotides on the gold nanoparticles have sequences capable of hybridizing with different portions of the sequence of a target nucleic acid, but not with each other. When a target nucleic acid comprising sequences complementary to the sequences of the oligonucleotides on the latex microspheres and gold nanoparticles is contacted with the two probes, a network structure is formed (see Figure 21). Due to the quenching properties of the gold nanoparticles, the fluorescence of the oligonucleotides attached to the latex microspheres is quenched while part of this network. Indeed, one gold nanoparticle can quench many fluorophore molecules since gold nanoparticles have very large absorption coefficients. Thus, the fluorescence of a solution containing nucleic acid and the two particles can be monitored to detect the results, with a reduction in, or elimination of, fluorescence indicating a positive result. Preferably, however, the results of the assay are detected by placing a droplet of the solution onto a microporous material (see Figure 21). The microporous material should be transparent or a color (*e.g.*,

white) which allows for detection of the pink/red color of the gold nanoparticles. The microporous material should also have a pore size sufficiently large to allow the gold nanoparticles to pass through the pores and sufficiently small to retain the latex microspheres on the surface of the microporous material when the microporous material is washed. Thus, when using such a microporous material, the size (diameter) of the latex microspheres must be larger than the size (diameter) of the gold nanoparticles. The microporous material must also be inert to biological media. Many suitable microporous materials are known in the art and include various filters and membranes, such as modified polyvinylidene fluoride (PVDF, such as Durapore™ membrane filters purchased from Millipore Corp.) and pure cellulose acetate (such as AcetatePlus™ membrane filters purchased from Micron Separations Inc.). Such a microporous material retains the network composed of target nucleic acid and the two probes, and a positive result (presence of the target nucleic acid) is evidenced by a red/pink color (due to the presence of the gold nanoparticles) and a lack of fluorescence (due to quenching of fluorescence by the gold nanoparticles) (see Figure 21). A negative result (no target nucleic acid present) is evidenced by a white color and fluorescence, because the gold nanoparticles would pass through the pores of the microporous material when it is washed (so no quenching of the fluorescence would occur), and the white latex microspheres would be trapped on top of it (see Figure 21). In addition, in the case of a positive result, changes in fluorescence and color can be observed as a function of temperature. For instance, as the temperature is raised, fluorescence will be observed once the dehybridization temperature has been reached. Therefore, by looking at color or fluorescence as a function of temperature, information can be obtained about the degree of complementarity between the oligonucleotide probes and the target nucleic acid. As noted above, this detection method exhibits high sensitivity. As little as 3 femtomoles of single-stranded target nucleic acid 24 bases in length and 20 femtomoles of double-stranded target nucleic acid 24 bases in length have been detected with the naked eye. The method is also very simple to use. Fluorescence can be generated by simply illuminating the solution or microporous material with a UV lamp, and the fluorescent and colorimetric signals can be monitored by the naked eye.

Alternatively, for a more quantitative result, a fluorimeter can be employed in front-face mode to measure the fluorescence of the solution with a short pathlength.

The above embodiment has been described with particular reference to latex microspheres and gold nanoparticles. Any other microsphere or nanoparticle, having the other properties described above and to which oligonucleotides can be attached, can be used in place of these particles. Many suitable particles and nanoparticles are described above, along with techniques for attaching oligonucleotides to them. In addition, microspheres and nanoparticles having other measurable properties may be used. For instance, polymer-modified particles and nanoparticles, where the polymer can be modified to have any desirable property, such as fluorescence, color, or electrochemical activity, can be used. See, Watson et al., *J. Am. Chem. Soc.*, **121**, 462-463 (1999) (polymer-modified gold nanoparticles). Also, magnetic, polymer-coated magnetic, and semiconducting particles can be used. See Chan et al., *Science*, **281**, 2016 (1998); Bruchez et al., *Science*, **281**, 2013 (1998); Kolarova et al., *Biotechniques*, **20**, 196-198 (1996).

In yet another embodiment, two probes comprising metallic or semiconductor nanoparticles having oligonucleotides labeled with fluorescent molecules attached to them are employed (illustrated in Figure 22). The oligonucleotide-nanoparticle conjugates can be prepared and labeled with fluorescent molecules as described above. The oligonucleotides on the two types of oligonucleotide-nanoparticle conjugates have sequences capable of hybridizing with different portions of the sequence of a target nucleic acid, but not with each other. When a target nucleic acid comprising sequences complementary to the sequences of the oligonucleotides on the nanoparticles is contacted with the two probes, a network structure is formed (see Figure 22). Due to the quenching properties of the metallic or semiconductor nanoparticles, the fluorescence of the oligonucleotides attached to the nanoparticles is quenched while part of this network. Thus, the fluorescence of a solution containing nucleic acid and the two probes can be monitored to detect the results, with a reduction in, or elimination of, fluorescence indicating a positive result. Preferably, however, the results of the assay are detected by placing a droplet of the solution onto a microporous